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Large-Scale Analyses of the Relationship between Sex, Age, and Intelligence Quotient Heterogeneity and Cortical Morphometry in Autism Spectrum Disorder

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Abstract

Significant heterogeneity across aetiologies, neurobiology, and clinical phenotypes have been observed in individuals with autism spectrum disorder (ASD). Neuroimaging-based neuroanatomical studies of ASD have often reported inconsistent findings which may, in part, be attributable to an insufficient understanding of the relationship between factors influencing clinical heterogeneity and their relationship to brain anatomy. To this end, we performed a large-scale examination of cortical morphometry in ASD, with a specific focus on the impact of three potential sources of heterogeneity: sex, age and full-scale intelligence (FIQ). To examine these potentially subtle relationships, we amassed a large multi-site dataset that was carefully quality controlled (yielding a final sample of 1327 from the initial dataset of 3145 magnetic resonance images; 491 individuals with ASD). Using a meta-analytic technique to account for inter-site differences, we identified greater cortical thickness in individuals with ASD relative to controls, in regions previously implicated in ASD, including the superior temporal gyrus and inferior frontal sulcus. Greater cortical thickness was observed to be sex-specific; further, cortical thickness differences were observed to be greater in younger individuals and in those with lower FIQ, and to be related to overall clinical severity. This work serves as an important step towards parsing factors that influence neuroanatomical heterogeneity in ASD and is a potential step towards establishing individual-specific biomarkers.

Introduction

Early brain overgrowth was one of the earliest neural phenotypes reported in autism spectrum disorder (ASD)[1,2]. However, subsequent studies examining advanced cortical phenotypes have reported diverse and conflicting neuroanatomical findings. For example, increases as well as decreases have been reported in both cortical thickness[3–6] and surface area[7–10]. This may, in part, be attributable to factors that influence phenotypic heterogeneity in ASD, such as age, sex, and intelligence[11–14]. However, these potential sources of heterogeneity are commonly regressed out as nuisance variables in statistical modelling or not considered in ASD studies. In the face of limited sample sizes, previous studies have omitted females altogether[5,15,16], or examined limited age ranges[8,9,17–19], while others typically do not examine associations with intelligence. The limited studies considering these factors have observed that ASD-related atypical neuroanatomy varies greatly by age[5,20–25], sex (see Lai et al[26] for a review) and estimated intelligence[27], suggesting a need to reconcile the association between factors that contribute to clinical heterogeneity and neuroanatomical differences. It is also possible that previous findings may be further confounded by biases in morphological estimates related to movement during image acquisition (particularly given the observation that neurotypical and males with ASD are most likely to move during scanning)[28,29], and variations in the quality control of image processing outputs[30].

Here we sought to reconcile the impact of sex, age, and estimated intelligence on heterogeneity in ASD cortical morphology by performing a large-scale neuroimaging study using magnetic resonance imaging data acquired from multiple sources (initial dataset of 3145 subjects, 1327 subjects after rigorous quality control).

Based on previous findings reported in the literature, we expected to see overall greater cortical thickness in individuals with ASD relative to neurotypical controls [5,31,32]. Given known clinical, behavioural, and neuroanatomical sex differences in ASD, we expected these differences to differ in regional composition by sex [26,33,34]. We also expected these differences to be more pronounced in younger [5,35] and lower IQ individuals [17,27].

Methods

Sample. Cross-sectional data included here were acquired from previous studies by the National Institute of Mental Health (USA), the Hospital for Sick Children (Canada), the Cambridge Family Study of Autism (UK), and the UK Medical Research Council Autism Imaging Multicentre Study (UK MRC-AIMS). We also included publicly available data from the Autism Brain Imaging Data Exchange (ABIDE) I and II.[36,37]

The total initial sample size amounts to 3145 individuals: 1415 individuals with ASD (1165 male/250 female) and 1730 controls (1172 male/558 female), aged 2-65 years. See supplementary methods section S1 “Sample details” for imaging parameters and participant demographics.

Quality control and site elimination. Rigorous quality control (QC) was performed by two independent raters (SB, and either ST or MMC) at both the level of the raw input images (for motion and scan quality), and on processed outputs (see supplementary methods section S2 “Quality control and site elimination”; supplementary figures S1 and S2). Sites with three or more individuals per sex and diagnostic group remaining after QC were included (final dataset of 1327 individuals; 491 individuals with ASD (362 male/129 female) and 836 neurotypical controls (481 male/355 female) (supplementary tables S1 and S2). All analyses, unless otherwise indicated, were performed using this dataset of 1327 individuals.

Image processing. All T1-weighted images were pre-processed using the minc-bpipe-library pre-processing pipeline (https://github.com/CobraLab/minc-bpipe-library), and then submitted to the CIVET processing pipeline[38] (version 1.1.12; Montreal Neurological Institute), to estimate cortical thickness, surface area, and volume. Image processing and quality control was standardised across all data, and conducted within a single laboratory. For details, see supplementary methods sections S3 “Image pre-processing” and S4 “Image processing”.

Statistical analysis

To account for differences in scanners, acquisitions, and sample characteristics, statistical analysis was conducted using a prospective meta-analytic technique, where each site is initially treated as an independent study and results are pooled to define significance (see van Erp et al.[39]). First, multiple linear regressions were conducted to derive per-site Cohen's *d* effect sizes for the main effect of each variable of interest. An aggregate statistic representing all sites was derived by pooling effect sizes in a random-effects meta-analysis[40,41] (*metafor* 2.0-0 package in R 3.4.0). For examples of statistical models employed, see supplementary methods section S5 "Statistical models used".

Code availability

R code used to conduct the prospective meta-analyses described here is available from the corresponding authors upon request.

Case-control comparisons: global measures

Differences in mean cortical thickness (CT), total surface area (SA), cortical volume (CV), total grey matter (GM), total white matter (WM) and total brain volume (TBV) were compared between individuals with ASD and controls by examining the main effect of diagnosis, while including age (linear term) and sex in the model. Results were Bonferroni corrected with $p<0.008$ (based on 6 tests) being considered significant. GM and WM analyses were reanalyzed while controlling for TBV, to determine if these were differentially affected when accounting for global measures.

Case-control comparisons: vertex-wise analysis

Regional alterations in CT and SA were examined using the same meta-analytic technique and model described above for global measures, but extended to a vertex-wise level (81,924 vertices across the brain), and corrected for multiple comparisons using the false discovery rate [FDR][42]. To control for multiple comparisons both across vertices and across the various analyses done, p-values from all vertices

of all main analyses were pooled (including each interval of the age- and FIQ-centred analyses described in the subsequent sections), and a 5% FDR threshold was used to control for multiple comparison across all statistical tests conducted. This stringent FDR correction was applied separately for cortical thickness and surface area analyses.

Case-control comparisons were also examined using a mixed-effects model with site as a random factor to determine if our results diverge from previous large-scale studies that used this methodology (e.g. van Rooij et al[6]).

Heterogeneity-focused analyses: Importance of sex, age and FIQ

To assess the significance of sex, age and FIQ in our vertex-wise analysis of cortical alterations, we fitted two models for each variable: one including the variable of interest (i.e. sex, age, or FIQ), plus an interaction term between that variable and diagnosis, and the other without the variable of interest, or the interaction, in the model. Please see supplementary methods section S5 "Statistical models used" for details. We then used Akaike information criterion[43] (AIC, representing the best model fit) to determine the importance of the variable at each vertex, within each site separately. At each vertex, we determined the number of sites for which each model was shown to be the best fit, and calculated a weighted average (based on site size) to determine the best model, on average, at that vertex, taking into account all sites.

Based on the AIC comparison of the models, sex, age, and FIQ were demonstrated to be important explanatory variables at a substantial proportion of vertices across the brain for both CT and SA, motivating our further examination of these factors and their impact on cortical alterations in ASD (see supplementary figure S3 and S4).

Sex-focused analyses

Sex specific patterns were examined using the case-control analysis described above separately in males and females (for global and vertex-wise measures), with diagnosis and age (linear term) included in the model.

Age-focused analyses

Given variable reporting of best-fit trajectories in ASD and typical neurodevelopment in general[30,44], we tested the best model fit between linear, quadratic and cubic models of age (all models also included diagnosis, each age term, interaction between diagnosis and each age term, and sex; see supplementary methods section S5 for statistical models used). To do this, at each vertex, the minimum AIC was determined for each site, and a weighted average across sites was calculated per vertex, as described above.

The AIC for age revealed the linear model to be the best fit at most sites (range across vertices: 22-100% of sites) across most of the cortex for both cortical thickness (supplementary figure S5A) and surface area (supplementary figure S6A).

Next, an age-centered analysis was used to examine age-dependent changes in patterns of vertex-wise CT and SA alterations by centering age at intervals of 2 years, *accounting for age as a linear term.* This allows us to illustrate the differential effects on cortical thickness at different ages, and allows interpretation of group differences at the centered age interval. Essentially, this provides a "snapshot" of the groups' regression lines at that interval, without having to split the dataset into age ranges, thereby maximising power, and case-control differences were examined at each age interval[5,45]. This was done by calculating the per-site Cohen's d effect size for the main effect of diagnosis from each model (each age interval), and pooling these effect sizes in the random effects meta-analysis in the same manner as the case-control comparisons.

FIQ-focused analyses

The best model fit for the FIQ analyses was tested in the same way as the age analyses described above: the best model fit was tested between linear, quadratic and cubic models of FIQ (all models also included diagnosis, each FIQ term, interaction between diagnosis and each FIQ term, age and sex).

The AIC for FIQ revealed the linear model to be the best fit at most sites (range across vertices:

24-100% of sites) across most of the cortex for cortical thickness (supplementary figure S5B) and surface area (supplementary figure S6B).

An FIQ-centered analysis was performed in the same fashion as the age-centered analysis, with FIQ centered at intervals of 10 points, *and using a linear term for FIQ*. Results are examined at intervals of FIQ=80 and above, as there are very few controls with an FIQ<80. As FIQ data were not available for all individuals, this analysis was performed on a slightly smaller subset of 1214 individuals.

Associations between cortical thickness and ASD symptoms/characteristics

As consistent autistic symptom or characteristics measures were not available across all sites, analyses were performed on subsets of individuals who had the same measures, as in previous studies[6,46]. We chose the measures which had the largest number of individuals available, which included the ADOS-2 Calibrated Severity Scores (CSS)[47] to examine overall symptom severity (N= 279; also conducted separately in males [N=224] and females[N=55]), the ADOS-G reciprocal social interaction domain score, communication domain score, and restricted, repetitive behaviour [RRB] domain score (module 4; N=151), and the ADOS-2 RRB domain score and social affect domain score (module 3; N=143), all in individuals with ASD only. In both ASD and control individuals, we examined associations between CT and scores of the Social Responsiveness Scale (SRS; N=413) and Autism Spectrum Quotient[48] (AQ; N=171), as well as their interaction with diagnosis.

These analyses were conducted using a meta-regression technique. See supplementary methods section S6 “Associations between cortical thickness and ASD severity and symptoms” for meta-analysis details and subset sample characteristics.

Finally, we also performed a separate analysis to examine the potential effects of comorbid diagnoses on cortical alterations related to ASD. We repeated the case-control analysis excluding data from individuals with comorbid diagnoses (limiting our analyses to sites with this type of data recorded; resulting in a dataset of N=519; 144 ASD/375 Controls).

Case-control, sex-stratified and age centered analyses including FIQ in the model

Based on the results of the AIC analysis assessing the importance of FIQ as an explanatory variable, we examined the diagnosis, age-centered and sex-stratified analyses including FIQ in the model. This was done in the subset of individuals for whom FIQ data were available (N=1214). For these analyses, FDR correction was conducted across all analyses (including all age intervals) together, but separately from the main set of analyses.

Impact of quality control (QC)

We examined the impact of quality control on both the neuroanatomy and demographics of our sample (see supplementary methods section S7 “Quality control analysis”).

Power calculation

We used G*Power version 3.1.9.4, to determined the minimum detectable effect sizes given our sample size of 491 individuals with ASD and 836 controls. At a power level of 0.8 and a significant threshold of 0.05 (two-tailed), we determined we would be have the statistical power to detect effect sizes of 0.1463 and greater. However, this is based on a simple multiple linear regression analysis that pools all data together, ignoring the differences between sites, and not accounting for this in the analysis. It is unclear how the meta-analytic technique employed here would affect these estimates.

Results

Results of case-control comparisons and sex-focused analysis are presented in figure 1, the age-focused analysis in figure 2, the FIQ-focused analysis in figure 3, and symptom/severity-focused analysis in figure 4.

Greater cortical volume and mean and regional cortical thickness in ASD

We observed significantly greater CV ($p<0.008$; Cohen’s $d=0.17$) and mean CT ($p<0.0001$;

Cohen's d=0.22) and a trend towards enlarged TBV ($p<0.05$; Cohen's d = 0.11) in individuals with ASD (figure 1A). No differences were observed in total SA, WM or GM. When controlling for TBV, both GM and WM remain non-significant, however WM seemed to be slightly more affected, changing from p=0.1, to p=0.9 when controlling for TBV, whereas GM was barely affected (p=0.25 in original analysis, and p=0.24 when controlling for TBV).

In the vertex-wise analysis, regional group differences of CT (greater CT in ASD compared to controls) were observed in the inferior frontal and prefrontal cortex, superior temporal, postcentral, and posterior cingulate gyri and precuneus, bilaterally, surviving 5% FDR (peak Cohen's d = 0.32). Effect sizes showed some variability by site, however were largely positive (figures 1B and 1D; supplementary figure S7). The mixed effects model yielded similar results to the meta-analytic approach, however the results were less significant and over a smaller proportion of the cortex (supplementary figure S8). No significant differences were observed in SA.

Sex-specific cortical alterations

ASD males had significantly greater CV ($p<0.008$; Cohen's d=0.19) and mean CT ($p<0.008$; Cohen's d=0.21) compared to male controls (supplementary figure S9). WM volume trended towards being greater in ASD males relative to controls ($p<0.05$; Cohen's d=0.18). No differences in total SA or GM volume were observed. In females with ASD, mean CT trended towards being greater compared to controls ($p<0.05$; Cohen's d=0.21). No differences were observed in TBV, total SA, CV, GM or WM in the females (supplementary figure S10).

Both males and females with ASD presented with regions of significantly greater CT relative to controls, surviving 5% FDR, however, the observed patterns of CT differences were distinct between males and females (figure 1E and 1F). In ASD males, regions of greater CT were observed in bilateral superior temporal, inferior frontal, and right precentral gyri (peak Cohen's d = 0.39). In ASD females, these differences were observed in bilateral prefrontal and occipital cortices, and left posterior parietal cortex and pre- and postcentral gyri (peak Cohen's d = 0.45). Sex-specific effect sizes were overall

stronger than in the combined sample, and larger effect sizes were observed in the females compared to males (supplementary figures S11 and S12).

In the both males and females, for cortical thickness, the mixed effects model yielded similar but less diffuse results, and only survived 5% FDR in the left hemisphere (for both, see supplementary figure S13).

No significant differences in SA were observed in the males or females (meta-analytic model used for both).

Subtle age-specific cortical alterations

In the age-centered analyses, subtle but significant group differences in CT were maximal in childhood (8-10 years), with individuals with ASD presenting demonstrating greater CT relative to controls in small regions of the cortex. Figure 2 shows differences between individuals with ASD and controls at age intervals of four years, accounting for age using a linear model. Foci of significance were most apparent in the age range of 8-12 years, but the linear fits suggested steadily larger effect sizes for diagnosis on CT as one moves towards younger ages. Between the ages of 6-14 years, regions of significantly greater CT were observed primarily in lateral temporal and frontal regions, and the posterior cingulate cortex. After 12-14 years, less difference is observed between groups, and these differences were observed only in medial prefrontal regions.

In the age centered surface area analysis, no significant differences in SA were observed at any age interval.

FIQ

Individuals with ASD with lower FIQ were observed to have much greater and more widespread differences in CT relative to controls than those with higher FIQ (figure 3), spanning large regions of the frontal, temporal and occipital cortices. Foci of significance were most apparent in the FIQ range of 100-100, but the linear fits suggested steadily larger effect sizes for diagnosis on CT as one moves towards

lower FIQ. At FIQ of 120 only minimal significant group differences in CT are observed. Higher than this, no significant differences are seen.

In the FIQ centered surface area analysis, no significant differences in SA were observed at any FIQ interval.

Associations between cortical thickness and ASD symptoms/characteristics

A significant, positive correlation between CT and ADOS-2 CSS was observed in ASD individuals, primarily in the right hemisphere. This relationship was observed in regions in which individuals with ASD presented with significantly greater CT relative to controls, including the right superior temporal gyrus and inferior frontal sulcus, right orbitofrontal cortex, and bilateral posterior cingulate cortices. Furthermore, motivated by our findings of sex-specific regions of CT alterations in subjects with ASD, we explored the relationship between CT and CSS in males and females separately. In the female sample, we observed a significant positive relationship between CT and severity, primarily in prefrontal and temporal regions. Conversely, in the males, only very minimal regions showed this significant relationship, despite the much larger sample size compared to the females (figure 4). Males and females in this sample did not differ significantly in severity or FIQ.

No significant associations were observed between the SRS or AQ and CT. Only very minimal significant associations were observed for ADOS domain scores with CT, in very small cortical regions. Please see supplementary results section S7 and supplementary figures S14 and S15 “Associations between neuroanatomy and ASD symptoms/characteristics” for details.

Based on our analysis of the potential impact of comorbidities, including only individuals with ASD with no comorbid features does not seem to change the spatial extents of our results, but does impact the number of vertices surviving 5% FDR, and increases the overall effect size. Please see supplementary results section S8 and supplementary figure S16 for details.

Case-control, sex-stratified and age-centered analyses including FIQ in the model

Including FIQ in the model did not substantially alter the results for the diagnosis main effect, sex-stratified analyses, or age-centered analyses. Please see supplementary results section S9, and supplementary figures S17-S20.

Discussion

In this study we use a large dataset that has been strictly quality controlled and analyzed using harmonized image processing and statistical methods in order to study variation in cortical anatomy in ASD. Our results demonstrate greater cortical thickness in widespread cortical regions in individuals with ASD, primarily in the frontal and superior temporal cortex, as well as the precuneus and posterior cingulate cortices. Cortical alterations were observed to be differentially impacted by sex, age, and FIQ. Greater CT was observed in largely different regions between males and females, with females demonstrating potentially greater magnitude of cortical thickness alterations than males, relative to same-sex controls. Group differences were greatest in childhood, and differences lessen after early adulthood. Alterations were observed in largest regions and were more significant in individuals with FIQ of 80-110, with almost no significant group differences observed in individuals with FIQ of 120 and higher. In ASD individuals, greater CT was positively correlated with symptom severity measured by ADOS-2 CSS, in regions which also showed greater CT relative to controls, and these correlations were stronger, and seen in distinct regions, in females compared to males.

Greater total brain volume (TBV) in very young children with ASD is one of the most consistently reported findings in the ASD neuroimaging literature[49–51], and some studies show that this larger brain volume persists into adolescence[52]. Mechanisms potentially underlying increased TBV include increased neurogenesis, decreased synaptic pruning and neuronal cell death, and abnormal myelination[53]. Our results suggest that the larger TBV phenotype observed in ASD can also be recapitulated at levels of local and global cortical thickness (though here we only observed greater TBV

in ASD at a trend level). Increased cell proliferation in the ventricular zone during development has been suggested as underlying abnormalities in the number and width of cortical columns (resulting in increased cortical surface area), as well as increased neuronal density[54] (resulting in increased cortical thickness). Both cortical column abnormalities and increased neuronal density have both been reported in ASD[55], thus it is unclear why we do not observe the alterations in surface area in individuals with ASD reported by other studies[7–9]. It is also unclear how quality control may impact results (see below for further discussion on this). Thus this relationship warrants further investigation. Deficiencies in synaptic pruning[56], which begins in early life and continues into adolescence, have also been proposed as underlying the greater cortical thickness observed in ASD[57]. This is supported by studies reporting reduced synaptic pruning during development in children with ASD[58], and could explain the differences that persist into adulthood, as observed here.

It should be noted that other factors can affect cortical thickness measurements; for example, altered cortical myelination or reduced integrity of the gray-white matter boundary, potentially resulting from deficits in neuronal migration during early development. Specifically, this blurring of the cortical interface has been demonstrated in individuals with ASD in both histological post-mortem[59] and in-vivo neuroimaging studies[60,61], and could potentially lead to inaccuracies in cortical thickness estimates due to misplacement of the cortical boundary, with apparent increases in cortical thickness.

Previous studies[8,10,51] have reported very early expansion of the cortical surface and increased surface area in young children (2-5 years) and infants (6-24 months) with ASD, and suggest this may drive the early brain overgrowth that has been observed in ASD. In keeping with our results, other studies have found no group differences in SA in preschoolers[62], or children and adolescents[24]. However, lower SA has been observed in children with ASD aged 9-20 years, normalising in adulthood [9], as well as in a sample of male adults with ASD[7]. There is evidence that cortical thickness peaks around one or two years of age, and gradually decline thereafter into adolescence[63], whereas surface area develops rapidly in the first year of life, and continues to gradually expand into late childhood or adolescence, before

declining[63,64]. Therefore it is possible that the early increases in surface area in ASD observed in previous studies normalise after this period of rapid development, and thus were not captured in our sample, which has only very few individuals between the ages of 2-5.

With this large dataset, we hoped to reconcile some of the inconsistencies reported in the literature with regard to cortical phenotypes of ASD. While many other neuroimaging studies of ASD have reported greater cortical thickness values[5,15,31], others have reported lower thickness[65], or no differences[9]. Our findings of greater CT in ASD are largely in agreement with other large-scale neuroimaging studies, including studies using the ABIDE dataset[5,15,16,66] and recent findings by the ENIGMA consortium[6]. However, the recent ENIGMA study, in addition to greater CT in ASD in the frontal and posterior cingulate cortices, also reports significantly less CT in ASD in the temporal and parahippocampal cortices. We found no regions of significantly lower CT values; conversely, we observed greater CT, in multiple temporal regions. Methodological differences may account for the disparity between our results and those of the ENIGMA study, as well as others reporting decreased CT in ASD. These differences include our rigorous quality control (more discussion on this below), the analysis of region-specific differences using the vertex-wise extension of the prospective meta-analysis technique (instead of the regions of interest approach in the ENIGMA study), differences in image processing pipelines, and differences in sample characteristics (despite some overlap between our and the ENIGMA sample [ABIDE sample and ~150 controls from the Toronto sample]). Interestingly, the ENIGMA study found that the mixed-effect models strategy yielded more significant results than the meta-analytic technique, whereas we found the opposite. It is unclear how the choice of statistical method interacts with these other factors, however, we believe that the meta-analytic model better deals with the possible confounding variables and variability between sites, and that the mixed effects model may be less sensitive to capturing small effect sizes through the noise introduced by this variability.

Other studies using the ABIDE dataset have likewise found abnormalities in cortical thickness in ASD, in regions overlapping with our results, but of varying magnitude and direction[5,15,16]. Most

consistent between these studies is the observation of greater CT in individuals with ASD in the superior temporal gyrus, as well as frontal regions. However, it should be noted that most of these studies examine males only, and thus are more appropriately interpreted in comparison to our male-specific results.

Quality control likely greatly contributes to the inconsistencies in the literature; many studies do not describe their QC procedures in detail, rendering it difficult to assess the impact that motion or inaccurate segmentation may have on reported results. In our study, particular attention was given to motion artefact at the level of the raw input images, as in-scanner motion is known to cause apparent cortical thinning due to blurring of the grey-white matter boundary[28,67]. Thus, inadequate QC could lead to results of greater CT in individual with ASD, a population likely to move while being scanned, being attenuated or obscured by this effect. Importantly, in our sample, when no or minimal quality control was implemented, CT differences (greater in ASD) that were observed in the quality controlled sample were greatly attenuated. Additionally, regions of decreased CT in the bilateral temporal poles and left orbitofrontal cortex were also observed in individuals with ASD (supplementary figure S21). Decreased CT in these regions has previously been reported to be associated with motion[29,67], and these results highlight the potential for motion to confound results.

In addition to the issue of QC, it is often unclear to what extent case-control differences reported in the literature are influenced by factors contributing to the heterogeneity observed, such as age, sex, FIQ and severity[68]. Thus, another primary objective of this work was to begin to parse this heterogeneity observed in ASD, and determine to what extent these factors influence the reported diagnostic differences in neuroanatomy observed in previous studies, and the variability in these results. While these factors have been demonstrated to impact the neuroanatomical alterations in ASD[17,22,26], many studies do not take them into account when examining case-control differences.

In particular, the issue of sex differences in ASD has been receiving more attention recently, yet still studies examining neuroanatomical sex differences are rare, and have largely been underpowered due to small samples sizes of females with ASD[26]. Of existing studies examining sex differences in CT

specifically, results are varied: one such study found a sex-by-diagnosis interaction, with lower CT in ASD females, but greater CT in ASD males[34], [34] report no difference[6,33]. Even with our large sample and proportion of females with ASD (362 males and 129 females with ASD), we do not detect a significant sex by diagnosis interaction. However, when stratifying by sex, we demonstrate both qualitatively and quantitatively distinct diagnostic effects in males and females, as well as a sex-specific relationship between ASD symptom severity and cortical thickness. Our overall case-control results much more closely reflect those of the male-only findings, suggesting the female differences (observed in different regions, and with larger effect sizes) are obscured due to the small sample. Interestingly, the relationship between CT and ASD symptom severity seemed to be driven primarily by the females. This is in spite of the fact that in this sample, males and females do not differ significantly in ASD symptom severity or FIQ; suggesting that females perhaps need more substantial neuroanatomical alterations to result in the same level of clinical presentation as in males (in keeping with the female protective hypothesis[69,70]). These results highlight the importance of taking biological sex into account when studying ASD, as well as the urgent need for studies examining neuroanatomical sex differences in ASD in larger samples.

Age has been a significant contributor to the heterogeneity observed in ASD. Results of studies examining different age ranges of ASD, in particular in those with small sample sizes, are often conflicting or inconsistent. Recent large scale studies examining wide age ranges that have attempted to reconcile these inconsistencies have reported cortical thickness differences in childhood and early adolescence, followed by normalisation of group differences later in life[5,6,66]. While we cannot strictly make inferences about cortical development from our cross-sectional dataset, here, we seem to recapitulate these results to an extent, though the results observed in our age-centred analysis are subtle. This possible attenuation and eventual disappearance of diagnostic group differences in adolescence and adulthood could be the result of accelerated cortical thinning in ASD after an initial period of overgrowth, as has been observed in previous longitudinal samples[5,22] as well as post-mortem studies[71]. We also demonstrated a linear model to be the best fit for the majority of our dataset, across most of the cortex, as opposed to the curvilinear trajectories that have been reported by other studies[5,6] . This may be due, in

part, to the meta-analytic technique we chose to employ, which necessitated conducting the model fit on a per site basis, as some smaller sites may lack the power to model higher order trajectories. Improved quality control (QC) in our study may also play a role, as a recent study demonstrated that after strict QC, previously observed higher order trajectories were mostly replaced by linear effects[30]. While some early general population studies reported a peak in CT in late childhood followed by a decline[72,73], more recent studies, including those using generalised additive mixed models (GAMM)[74,75], have reported a monotonic decrease in CT from around two years of age[22,30,44,76,77]. Our findings, though cross-sectional, seem to support this reported linear decline in CT, rather than a peak later in childhood. Taken together, our findings may help further clarify the recent changes in our understanding of neurotypical and atypical cortical developmental trajectories[72,73] as these models continue to evolve in relation to the greater awareness of potential age-related biases related to motion and image processing quality control. However, given that our data are not longitudinal, and the inclusion of limited number of adults, these results should be interpreted with these caveats in mind. Larger, longitudinal studies will be necessary to confirm these findings.

Few studies have examined the potential moderating effects of IQ on the neuroanatomy of ASD, though there is some evidence suggesting that individuals with a diagnosis of Asperger's syndrome (with average or above average IQ) present with milder neuroanatomical atypicalities compared with lower IQ individuals[27,78]. Despite our sample being skewed to the cognitively higher functioning end of the spectrum, our results seem to align with these findings as we observed greater alterations in the lower FIQ part of our sample. Further, our observation of an inverse relationship between CT and FIQ in individuals with ASD, with the opposite or no relationship in controls, is aligned with previous studies of ASD[17] as well as in typically developing individuals.[79] Shaw et al.[80] also demonstrated that IQ is differentially associated with CT in children compared to adults; future larger-scale work should examine three way relationship between IQ, CT, and age in the context of ASD, as well as the extent to which group differences observed may be attributable to lower intellectual functioning rather than simply ASD diagnosis.

As ADOS versions and modules were not consistent across sites, we could not directly test the relations between region-specific cortical alterations and specific ADOS symptom domains in the whole sample. However, the positive relationship between ADOS-2 CSS and CT observed in a subset of individuals with ASD, in regions where case-control differences were observed, suggests a functional relevance of these cortical alterations. Some of the strongest group differences in both the overall sample and in the symptom-based analyses were observed in the superior temporal gyrus (STG) and inferior frontal gyrus (IFG) and might reflect the social communication deficits that are characteristic of ASD.[81–84] Interestingly, the IFG and STG were also the regions where the strongest case-control differences in CT were observed in males, but not in females.

The results presented here should be interpreted with respect to several limitations. Firstly, in order to amass the significant amount of data presented here, we were required to pool already collected data from multiple sites. The lack of standardization across sites of MRI acquisition, inclusion criteria, and clinical assessments between sites should be considered. While the meta-analytic statistics used pool common effect sizes across sites, the impact of this lack of standardization will certainly have an impact on our results. The lack of standardised measures across sites made examination of heterogeneity associated with specific ASD symptoms challenging. As a result, the impact of important factors such as socioeconomic status and parental education (which were not available for any of our sample) could not be ascertained. *Similarly, we could not directly assess the impact of specific* comorbid *diagnoses* (which were collected and coded inconsistently between sites); *however, based on the results of our analysis including only individuals with no comorbidities, the inclusion of individuals with ASD with comorbid features did not seem to substantially impact our results, though this may have added further variability and attenuated the effect of group differences observed. More targeted investigations into the relationship between common ASD-specific comorbidities and the clinical and neurobiological heterogeneity commonly observed in ASD is necessary.* Please see supplementary tables S3 and S4 for details on clinical and demographic data available per site. The statistical analysis method itself may also, in turn, be limited in its ability to detect small effects within each site, as well as curvilinear relationship with age or FIQ in

the smaller samples.

In addition, there are two considerations which would have improved our ability to better understand factors impacting heterogeneity. The first is the absence of genetic data. While ASD is highly heritable, it has been associated with a diverse number of risk genes[85–87] and rare copy number variants.[88,89] These genotypes have been observed to impact the heterogeneity of ASD and require further consideration. The second is the use of longitudinal data to truly model intra-individual change over time to better define alterations in neuroanatomical trajectories.[45,90] It is possible, given the large sample size used, that we have partially overcome this limitation given that our results are consistent with at least one large, longitudinal study examining cortical development in ASD.[22] Nonetheless, further investigation with large longitudinal samples that include males and females are clearly needed.

Finally, further consideration of the demographics of our sample is needed when interpreting our findings. This includes being cautious regarding interpretation of findings in the part of the sample >30 years old, as this represents a smaller subset of the study cohort. Second, the unbalanced male/female distribution requires further consideration. It is likely that we are only detecting the largest effect size differences between ASD and control females and there are likely smaller effects that we are underpowered to detect. Finally, individuals excluded due to QC were younger, had lower IQ and higher severity scores, and included a higher proportion of male and ASD individuals; thus biasing and further skewing our sample towards higher IQ individuals (see supplementary table S5). We acknowledge that smaller studies might not have the option of excluding such a large proportion of their data. However, in light of the potential contribution of motion and data quality to inconsistencies in the literature, there are certain steps that should be taken to ensure proper quality, and thus reliability, of data. These include the use of prospective motion correction techniques such as vNavs volumetric navigators[91], the recruitment of larger samples with the knowledge that there may be a large proportion of data that could not be used in statistical analyses, to book sufficient scanner time so as to allow re-scanning where necessary, and, in the case of small samples, to augment the sample using publicly available or collaborator data for replication

purposes. The potential exploration and subsequent use of motion/quality scores as confounding variables in analyses could also be considered[92,93]. Our thorough and rigorous manual QC was initiated and performed prior to the availability of these kinds of methods, thus we have not included these methods in our analysis. Nonetheless, we believe that the final QC used in this sample is extremely thorough.

Our findings address limitations in the literature regarding cortical neuroanatomy in ASD by combining multiple datasets. Our sample of 1327 individuals allowed us to detect significant group differences in the whole sample, as well as to examine potential sources of heterogeneity in relation to sex, age and FIQ, and their impact on cortical alterations in ASD. These findings highlight the importance of taking into account factors contributing to the phenotypic heterogeneity in ASD when examining the neuroanatomy in a supervised manner[68], which could further our research of the neurobiology of ASD.

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Figure captions

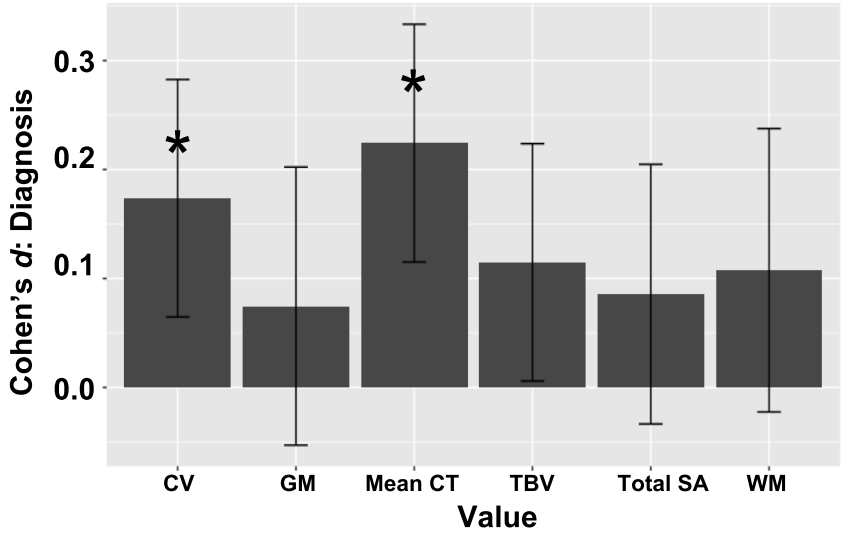
Figure 1. Case-control comparisons. Individuals with ASD presented with overall greater cortical volume and mean CT, and a trend towards greater total brain volume, as well as regionally specific differences in CT. These group differences are observed in sex-specific patterns of regional involvement, and are of a larger magnitude in the females. **A.** Cohen's d effect sizes for case-control comparisons of cortical volume (CV), total grey matter (GM), mean cortical thickness (CT), total brain volume (TBV), total surface area (SA) and total white matter (WM) (* denotes $p < 0.008$; error bars represent 95% confidence intervals). Positive effect sizes denote greater values in individuals with ASD compared to controls. Significantly greater CV ($p<0.008$) and mean CT ($p<0.0001$), are observed in individuals with ASD. **B.** Forest plot of Cohen's d effect sizes of mean CT per site. **C.** Significant vertex-wise group differences in CT across all subjects, shown at an FDR threshold of 5% (top), and effect size maps (bottom). Individuals with ASD show greater CT relative to controls. **D.** Forest plot showing effect sizes per site at a peak vertex in the right superior temporal gyrus. **E.** Significant vertex-wise group differences in CT in females, shown at an FDR threshold of 5% (top) and effect size maps (bottom). Females with ASD show greater CT relative to controls, primarily in left prefrontal, parietal and occipital regions. Effect sizes in females are greater than those seen in males. **F.** Significant vertex-wise group differences in CT in males, shown at an FDR threshold of 5% (top) and effect size maps (bottom). Males with ASD show greater CT relative to controls, primarily in bilateral inferior frontal and superior temporal regions.

Figure 2. Age-centered analysis. Main effect of diagnosis shown at 4 year intervals, using a linear model for age, shown at 5% FDR up until the age of 32, after which no significant differences are seen. Only minimal group differences are seen in the linear model, primarily in right superior temporal and inferior frontal regions. CT at a peak vertex in the left inferior frontal sulcus is plotted against age (bottom).

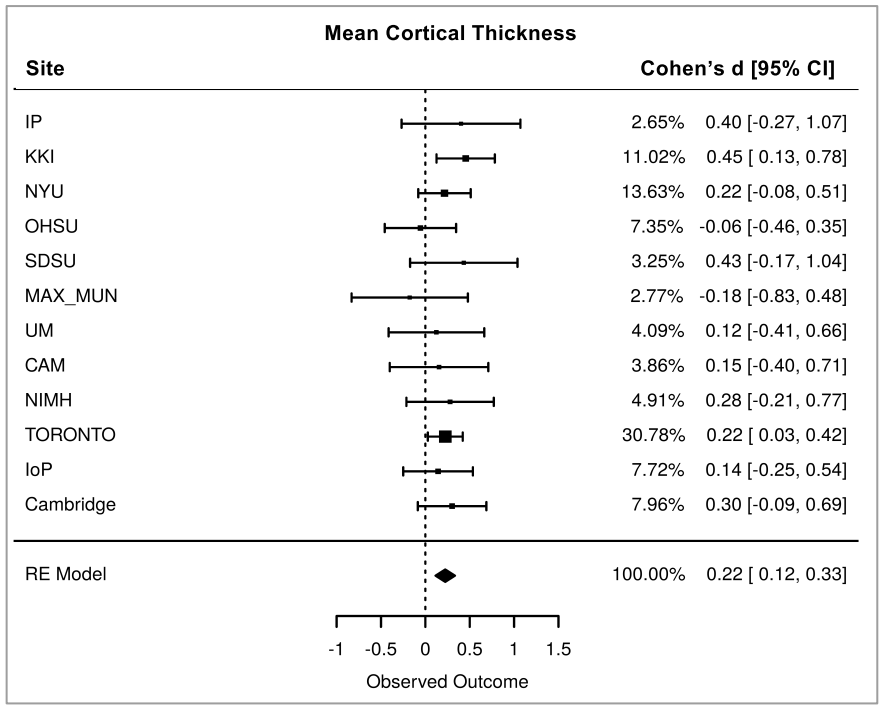
Figure 3. FIQ-centered analysis. Main effect of diagnosis at intervals of 10 FIQ points, using a linear model for FIQ (shown at 5% FDR), from an FIQ of 80, up until the age of 130, after which no significant differences are seen. Maximal differences were observed around an FIQ of 100. CT at a peak vertex in the right occipital lobe is plotted against FIQ (bottom).

Figure 4. Relation between CT and ADOS-CSS. Relationship between ADOS-2 calibrated severity scores (CSS) and CT in individuals with ASD, shown at a peak vertex in the inferior frontal sulcus (IFS). ADOS-2 severity was positively correlated with CT, primarily in the right hemisphere, in regions which show significant increases in individuals with ASD relative to controls. Correlations between CT and CSS were observed in distinct regions between males and females. In the female sample, there was a significant positive relationship between CT and severity, primarily in prefrontal and temporal regions. In the males, only very minimal regions showed this significant relationship, observed in the superior temporal gyrus and temporal pole. Shown at 5% FDR.

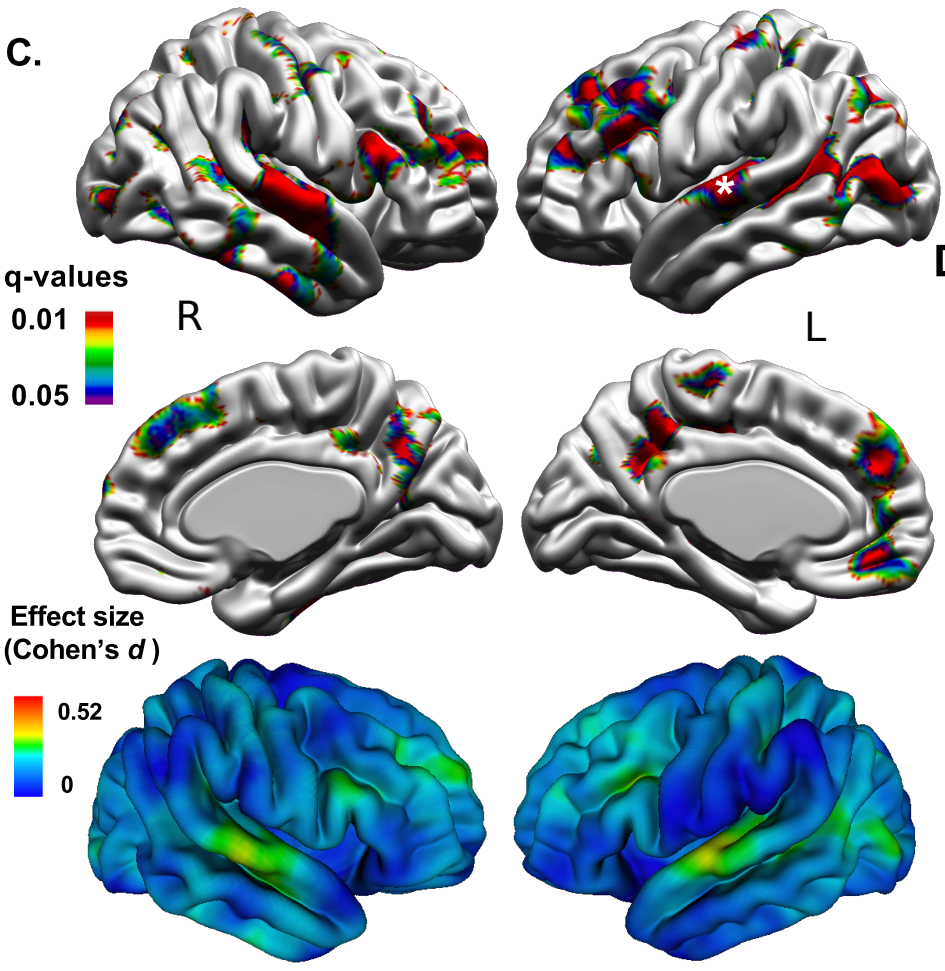
A.


Cohen's d: Diagnosis
0.3
0.2
0.1
0.0
*
*
CV
GM
Mean CT
TBV
Total SA
WM
Value

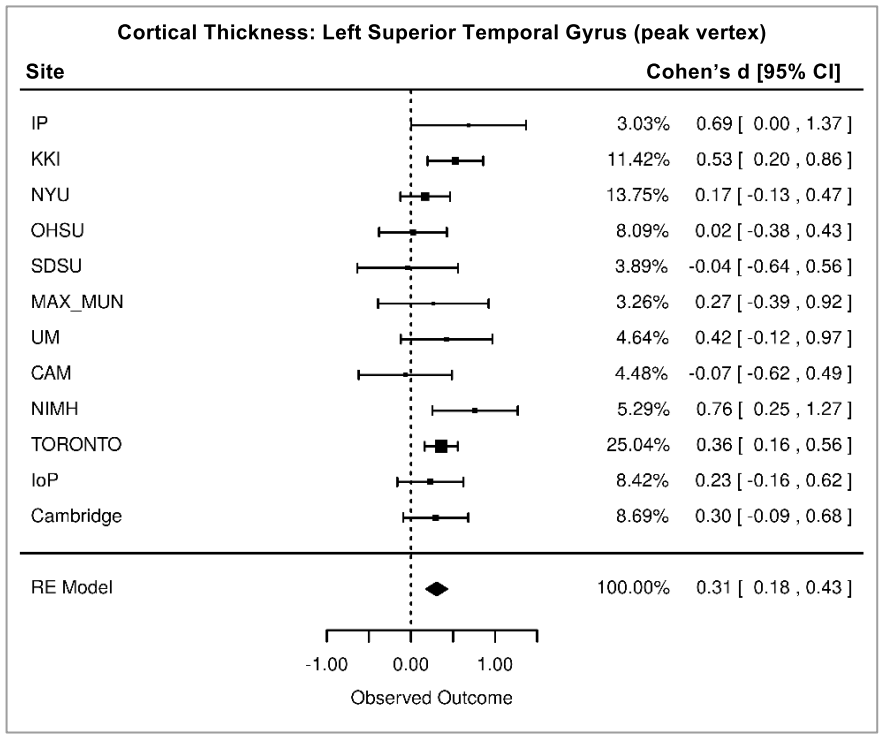
B.


Mean Cortical Thickness
Site
Cohen's d [95% CI]
IP 2.65% 0.40 [-0.27, 1.07]
KKI 11.02% 0.45 [0.13, 0.78]
NYU 13.63% 0.22 [-0.08, 0.51]
OHSU 7.35% -0.06 [-0.46, 0.35]
SDSU 3.25% 0.43 [-0.17, 1.04]
MAX_MUN 2.77% -0.18 [-0.83, 0.48]
UM 4.09% 0.12 [-0.41, 0.66]
CAM 3.86% 0.15 [-0.40, 0.71]
NIMH 4.91% 0.28 [-0.21, 0.77]
TORONTO 30.78% 0.22 [0.03, 0.42]
IoP 7.72% 0.14 [-0.25, 0.54]
Cambridge 7.96% 0.30 [-0.09, 0.69]
RE Model 100.00% 0.22 [0.12, 0.33]
-1 -0.5 0 0.5 1 1.5
Observed Outcome

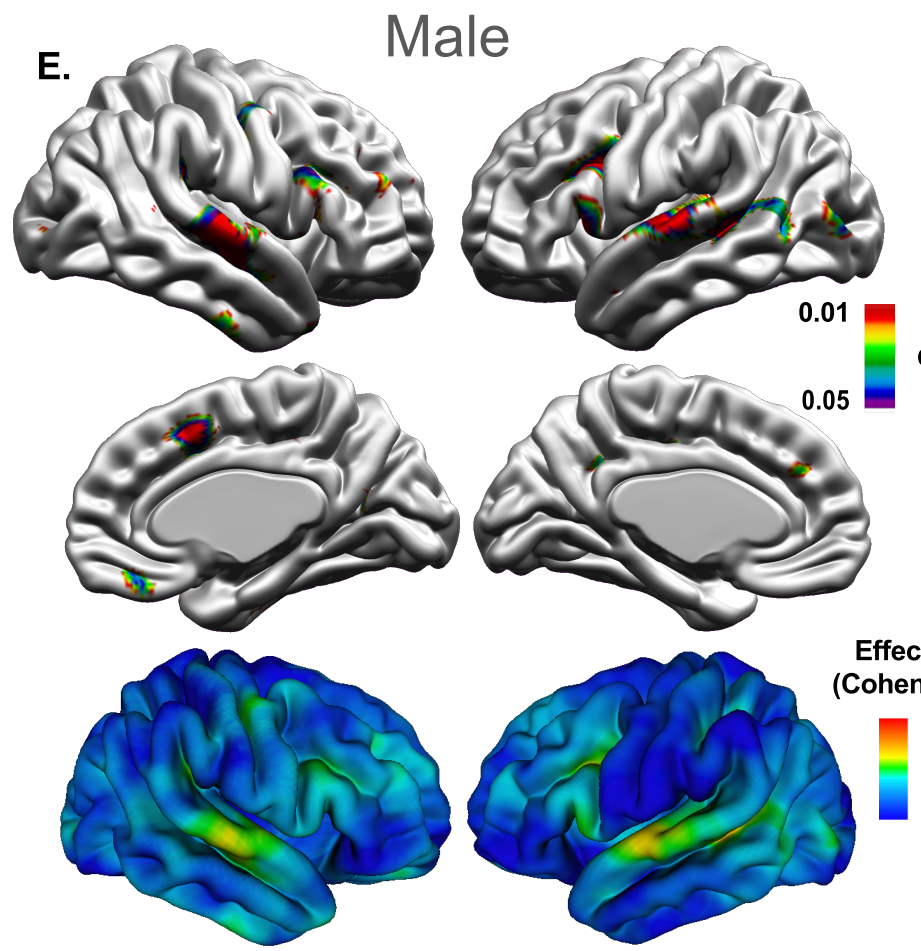
C.


q-values
0.01
0.05
R
L
Effect size
(Cohen's d)
0.52
0

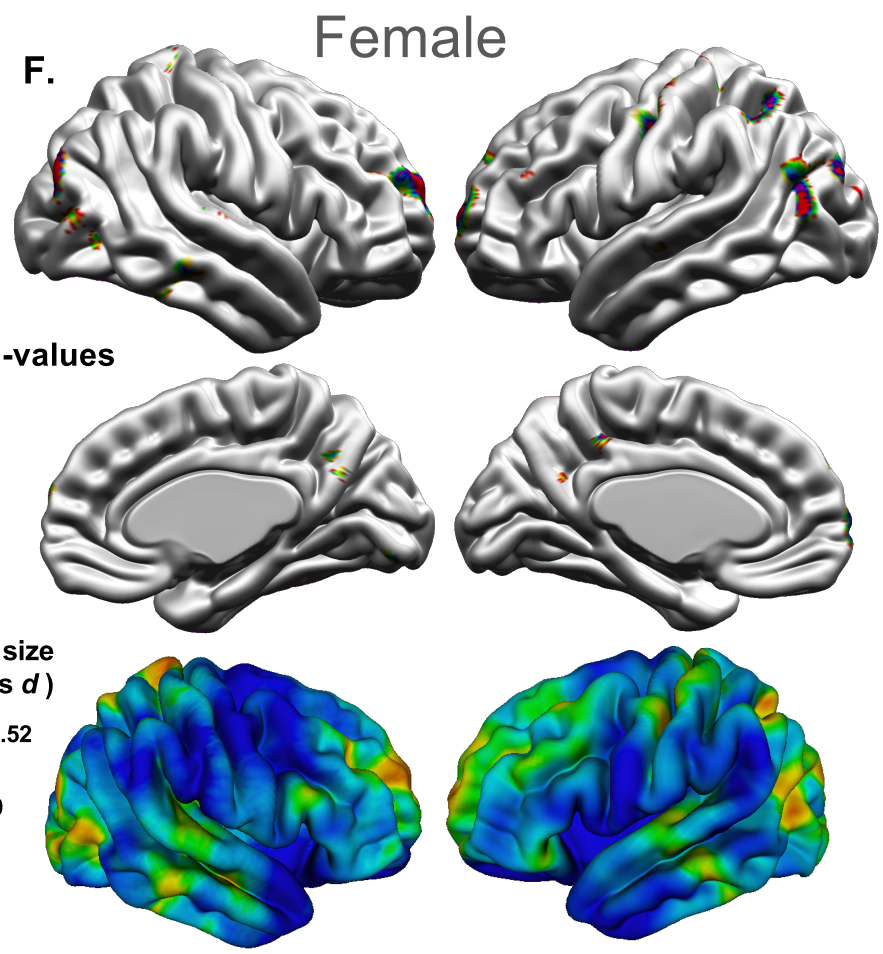
D.

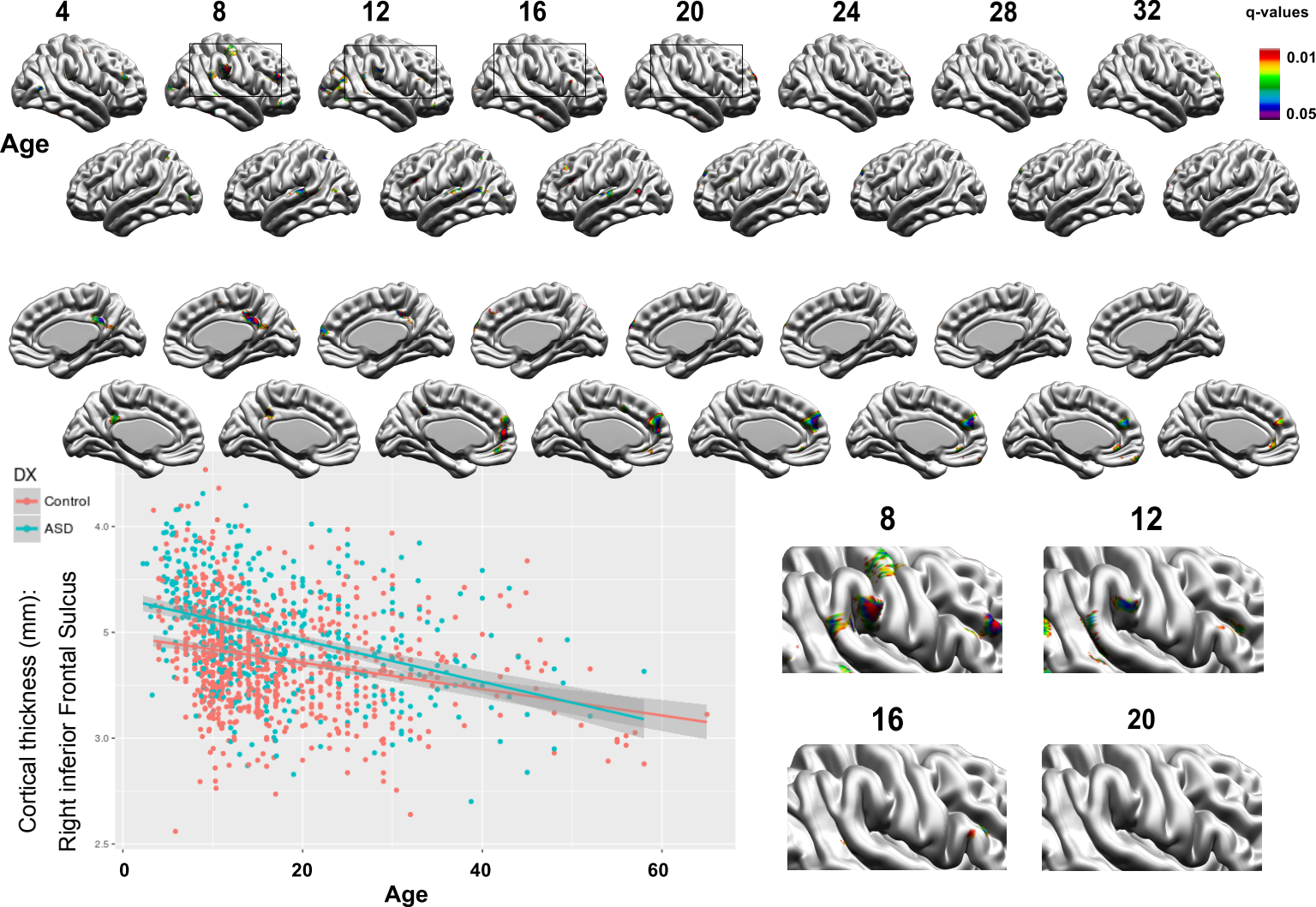

Cortical Thickness: Left Superior Temporal Gyrus (peak vertex)
Site
Cohen's d [95% CI]
IP 3.03% 0.69 [0.00 , 1.37]
KKI 11.42% 0.53 [0.20 , 0.86]
NYU 13.75% 0.17 [-0.13 , 0.47]
OHSU 8.09% 0.02 [-0.38 , 0.43]
SDSU 3.89% -0.04 [-0.64 , 0.56]
MAX_MUN 3.26% 0.27 [-0.39 , 0.92]
UM 4.64% 0.42 [-0.12 , 0.97]
CAM 4.48% -0.07 [-0.62 , 0.49]
NIMH 5.29% 0.76 [0.25 , 1.27]
TORONTO 25.04% 0.36 [0.16 , 0.56]
IoP 8.42% 0.23 [-0.16 , 0.62]
Cambridge 8.69% 0.30 [-0.09 , 0.68]
RE Model 100.00% 0.31 [0.18 , 0.43]
-1.00 0.00 1.00
Observed Outcome

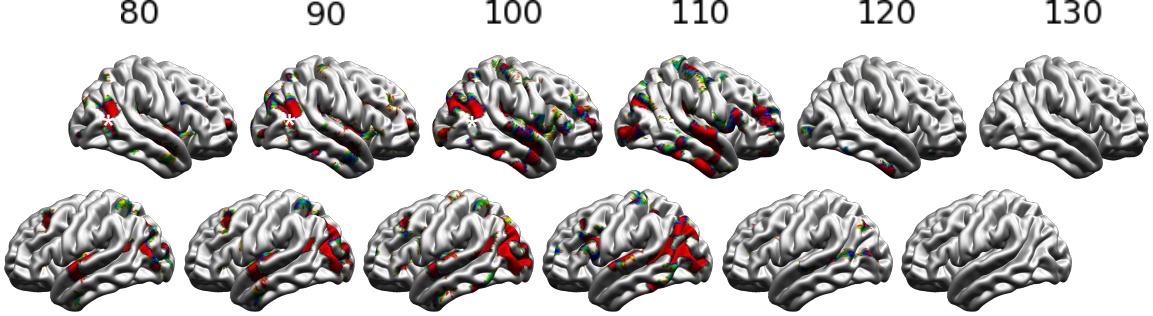
E.


Male
0.01
0.05
q-values
Effect size
(Cohen's d)
0.52
0

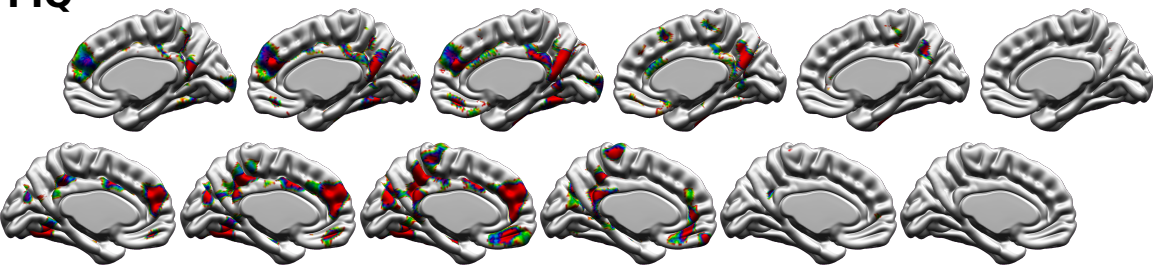
F.

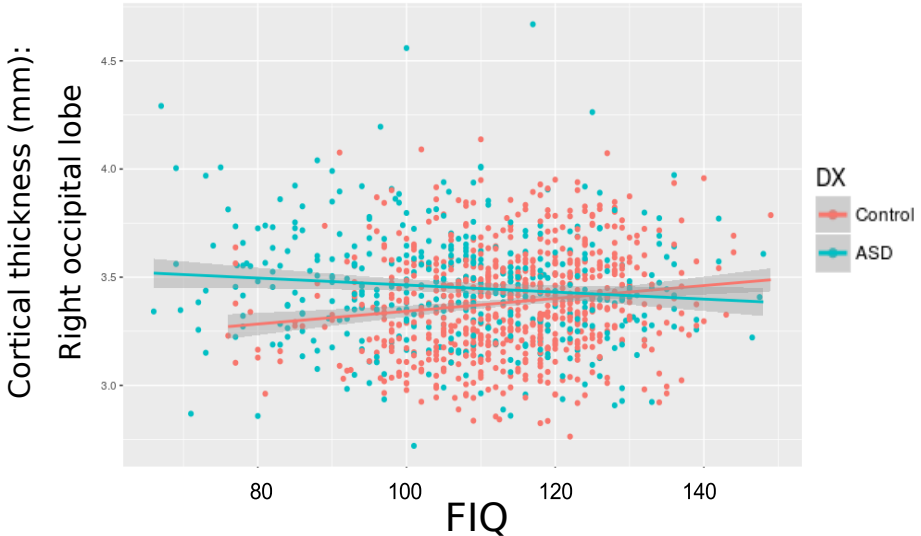

Female

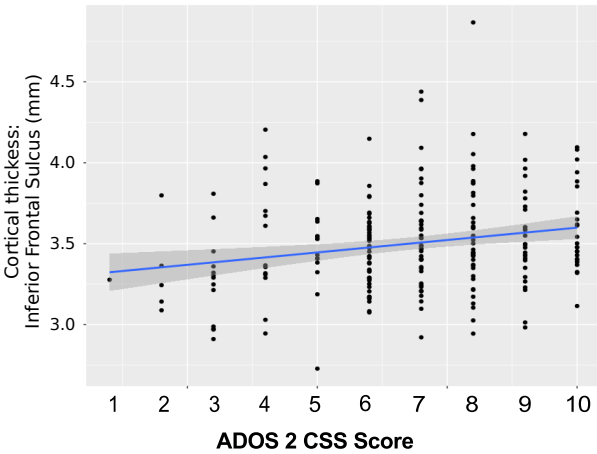

4
8
12
16
20
24
28
32
q-values
0.01
0.05
Age
DX
Control
ASD
Cortical thickness (mm):
Right inferior Frontal Sulcus
4.0
5
3.0
2.5
0
20
40
60
Age
8
12
16
20

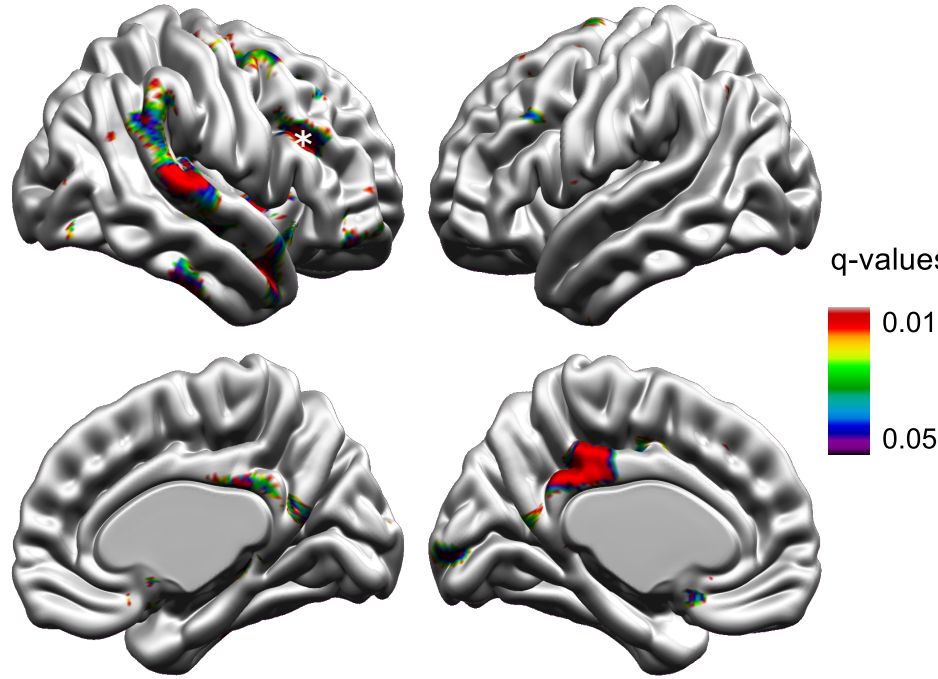

80
90
100
110
120
130

FIQ

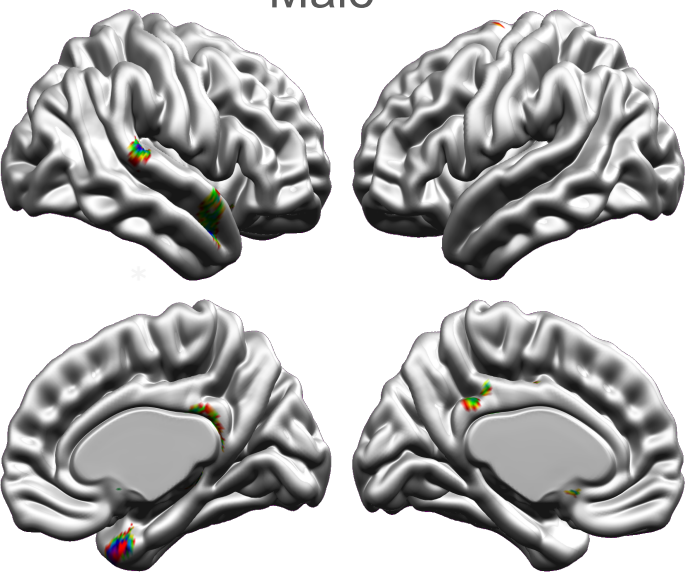



Cortical thickness (mm):
Right occipital lobe
4.5
4.0
3.5
3.0
80
100
120
140
FIQ
DX
Control
ASD

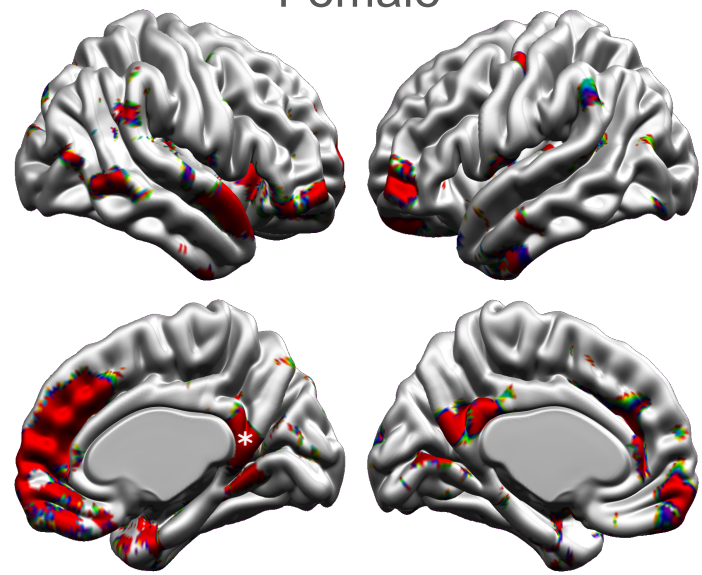

Cortical thickess:
Inferior Frontal Sulcus (mm)
4.5
4.0
3.5
3.0
1
2
3
4
5
6
7
8
9
10
ADOS 2 CSS Score

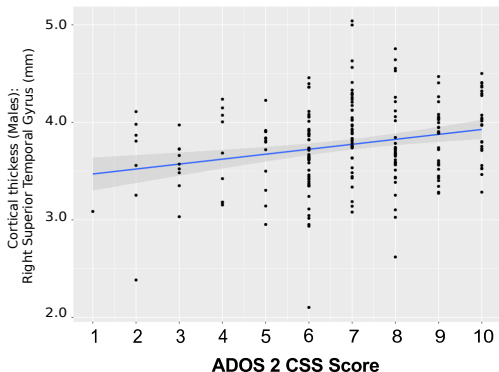

q-values
0.01
0.05

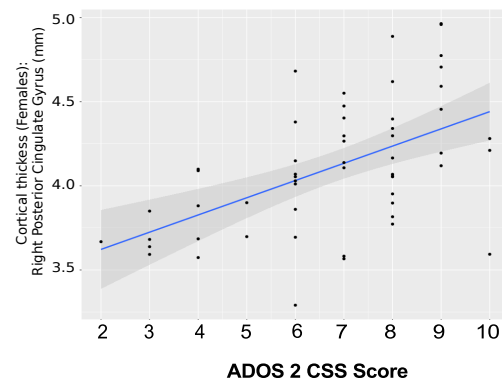
Male



Female




Cortical thickess (Males):
Right Superior Temporal Gyrus (mm)
5.0
4.0
3.0
2.0
1
2
3
4
5
6
7
8
9
10
ADOS 2 CSS Score


Cortical thickess (Females):
Right Posterior Cingulate Gyrus (mm)
5.0
4.5
4.0
3.5
2
3
4
5
6
7
8
9
10
ADOS 2 CSS Score